Partial Specific Volume of the Protein and Water in Beta-Lactoglobulin Crystals

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The partial specific volume of a protein is important in measuring protein solutions for determination of molecular size and shape[1] and for calculating the compositions of protein crystals from their densities.[2] In practice, specific volumes are calculated from density values. In calculating specific volumes of proteins, it is assumed that the density of the solvent, usually water, is unchanged by the protein. Because of this assumption, specific volumes determined at ordinary protein concentrations are referred to as apparent.

The specific volumes of the protein and water in β-lactoglobulin crystals reported in this paper were determined on crystals equilibrated at different relative humidities. The specific volumes were calculated from the densities and water contents of the protein crystals by the intercept method of Lewis and Randall.[3] When the water content of β-lactoglobulin crystals is between 14 and 46%, the value for the specific volume of the protein is 0.772 and that for the specific volume of the water is 0.983. These values may be compared with the value of 0.751 for the specific volume of this protein in solution if it is assumed that the value for the specific volume of the water is 1.003.

TABLE I

DENSITIES AND WATER CONTENTS OF β-LACTOGLOBULIN CRYSTALS AS A FUNCTION OF RELATIVE HUMIDITY AT 25°

Humidity Regulator	R.H., %	β-Lactoglobulin crystals	
		Water, %	Density
	0	0	1.247
LiBr (saturated)	6.3	2.39	1.252
LiCl (saturated)	12.0	3.07	1.253
H_2SO_4 (58.36%)	19.6	5.55	1.254
$MgCl_2$ (saturated)	31.6	7.24	1.254
H_2SO_4 (48.88%)	37.7	8.09	1.254
$Mg(NO_3)_2$ (saturated)	52.6	10.71	1.250
H_2SO_4 (37.76%)	61.0	12.90	1.248
NaCl (saturated)	75.3	16.01	1.241
KCl (saturated)	84.6	20.40	1.227
KNO_3 (saturated)	93.9	31.35	1.192
K_2SO_4 (saturated)	97.0	39.84	1.168
	100.0 (in water)	45.95	1.151

MATERIALS AND METHODS

β-Lactoglobulin Crystals

The previously described method for preparing large crystals was used.[4]

Composition and Densities of Crystals Equilibrated at Constant Relative Humidities

Completely hydrated, salt-free β-lactoglobulin crystals were placed in a series of desiccators containing saturated salt or sulfuric acid solutions (Table I) for maintaining constant humidities. The desiccators were evacuated to a pressure of 40 mm. of mercury and immersed in a water bath at 25 °C. At suitable intervals individual crystals were removed from the desiccators, and their densities and water contents were determined. The water content was determined by the previously described method.[5]

For determination of density, the equilibrated crystal was transferred rapidly to a density gradient tube made with xylene-bromobenzene saturated with water that had been calibrated with salt solutions of known densities.[6,7] After 5 minutes, the crystal came essentially to rest, and its density was interpolated between those of two drops of standard salt solutions, one having slightly higher density and the other having lower density. The values obtained for protein crystals by this method are reproducible to ± 0.001 in density.

The crystals were considered to be in equilibrium when the values obtained for density and water content did not change during two 3-day periods.

RESULTS

The results (Table I) for the densities and water contents of equilibrated crystals give smooth curves when plotted as a function of relative humidity (Fig. 1). The values for the water content as a function of relative humid-

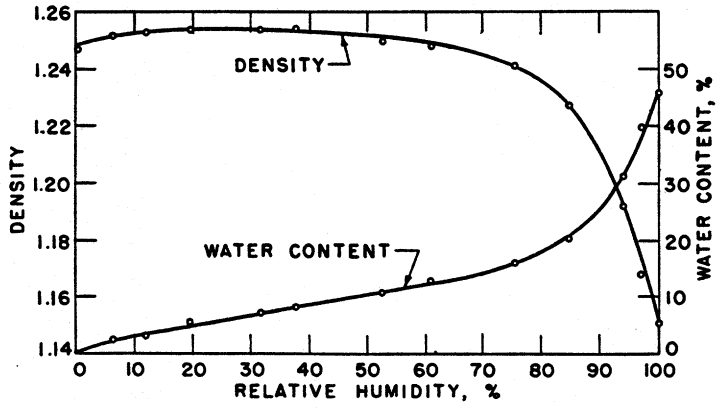

Fig. 1. Effect of relative humidity on the water content and density of β-lactoglobulin crystals.

ity are in good agreement with the values reported by Bull[8] for β-lactoglobulin crystals, and the shape of the curve[9,10] is similar to those of other proteins.

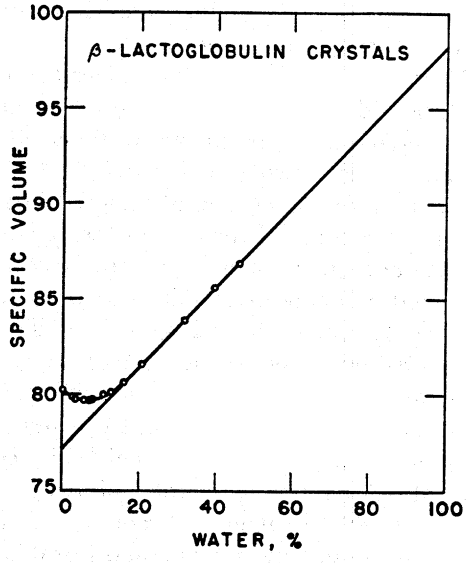

Fig. 2. Relationship between the specific volume and the water content of β-lactoglobulin crystals.

When the specific volumes ($1/d$) of the β-lactoglobulin crystals are plotted as a function of water content (Fig. 2) a straight line is obtained between 14 and 46% water. An extrapolation of this line, as required by the Lewis and Randall[3] graphical intercept method for determining partial specific volumes, gives a value of 0.772 for the specific volume of the protein and 0.983 for the specific volume of the water.

The following equation describes the straight-line portion of the curve that relates specific volume of the crystal to the water content (Fig. 2):

$$\text{Specific volume of crystal} = 0.002114 \times (\%\ \text{moisture}) + 0.7717$$

APPARENT VOLUME CHANGES OF β-LACTOGLOBULIN ON DISSOLVING AND ELECTROSTRICTION OF THE SOLVENT

The observed specific volume of anhydrous β-lactoglobulin is 0.802 as compared with the value of 0.772 in the crystals containing from 14 to 46% water (Fig. 2). This difference in volume of 0.030 ml. may be considered as an apparent packing of the protein due to the high density of the water molecules. Consistent with this idea is the finding of Katz[9] that the heat of swelling is large when small amounts of water are absorbed by a protein. It is also possible that the low value for the density of the protein containing small amounts of water is due to the presence of voids in the crystal

which the organic solvent used in measuring the density is not able to penetrate. The fact that finely divided proteins give the same type of contraction[11] as large crystal fragments, when small amounts of water are added, indicates that gross physical voids are not responsible for the apparent contraction and that the voids would have to be of molecular dimensions to account for this difference.

Chick and Martin,[12] who compared the density of proteins in the dry state and in solution, demonstrated that there is an apparent shrinkage of 5 to 8% in the volume of a protein when dissolved. In β-lactoglobulin the specific volume of the anhydrous protein is 0.802, whereas in solution it is 0.751[13], producing an apparent contraction of 0.051 ml. per gram protein. Cohn[14] has demonstrated that the electrostriction of solvent by the ionic groups of the protein is not sufficient to account for this contraction. If, however, the contraction be divided into apparent packing of the protein and contraction of the water, the volume contraction of the water is that expected to be caused by the electrostriction of the ionic groups in the protein. Thus the apparent packing of the protein is considered to be 0.030 ml. (0.802 − 0.772) and the contraction of the water to be 0.021 ml. (0.772 − 0.751). Cannan[15] found that β-lactoglobulin contains 58 carboxyl groups and 46 cationic groups, or a total of 104 ionic groups for an assumed molecular weight of 40,000. Accordingly, each gram of protein contains 0.0026 ionic group, and if Cohn and Edsall's[16] value of 10 ml. per charged group be used for electrostriction, a value of 0.026 ml. would be expected for the electrostriction due to ionic groups in one gram of protein. The difference between the observed value of 0.021 ml. and the calculated value of 0.026 ml. is considered to be within the errors involved in this calculation, and it is concluded that the ionic groups of the protein account for the contraction of the water.

DISCUSSION

Comparison of the results of Neurath and Bull[11] on the density of amorphous ovalbumin as a function of water content with similar data on β-lactoglobulin is of particular interest. When the results on ovalbumin are plotted, as in Figure 2, the curve is roughly parallel to the β-lactoglobulin curve, indicating that the forces holding the water in the amorphous ovalbumin are similar to those in the β-lactoglobulin crystal. The data on ovalbumin are not sufficiently precise, however, to give an extrapolated value for the partial specific volume of the ovalbumin and the water.

The values for the densities and the water content of β-lactoglobulin crystals (Fig. 1) form a smooth curve when plotted as a function of relative humidity and show no indication of a stepwise effect, as has been found for unit cell measurements of methemoglobin.[17] Perutz[2] has divided the water of hemoglobin crystals into "bound" and "free," based on the amount of salt and water in the crystal as compared with the concentration of salt in the suspending medium. "Bound" water is considered to be the same as

water of hydration, and a value of about 0.3 gram water per gram of hemoglobin was found in ammonium sulfate solutions. Briggs[18] has clearly stated the relation between the relative pressure-water content curve and the curve for "bound" water. If no combination of salt and protein occurs, it would be expected that "bound" water would vary with the vapor pressure of the solution and that the water content of β-lactoglobulin crystals as a function of vapor pressure (Fig. 1) would also be a measure of hydration of these crystals in salt solutions of equivalent vapor pressures.

Measurements of "bound" water in β-lactoglobulin crystals suspended in ammonium sulfate solutions gave results in good agreement with this hypothesis. Values of 0.29 to 0.22 gram of water per gram of protein were found for "bound" water when the concentrations of ammonium sulfate were varied between 2.7 to 4.0 molar with relative humidities from 88.7 to 81.8% as compared to values of 0.30 to 0.22 gram of water per gram of protein obtained for the corresponding relative humidities from the vapor pressure-water content curve (Fig. 1). The values reported for "nonsolvent" or "bound" water for β-lactoglobulin crystals suspended in sucrose solutions[4] are consistent with the idea that "bound" water varies with the vapor pressure of the solution. Thus "bound" water for β-lactoglobulin was found to vary from 0.27 to 0.49 gram of water per gram protein in solutions of sucrose with relative humidities from 87.3 to 99.6%. These values are lower than the values obtained from the vapor pressure-water content curve (Fig. 1). This difference in the values obtained by these two methods may be due to difficulties in making accurate measurements of hydration at high relative humidities or to the combination of sucrose with the protein.

This relation between the hydration of a protein from the vapor phase to that in solution has also been demonstrated by Adair and Robinson[19] who found a value of 0.2 gram water per gram of hemoglobin for vapor phase water absorption at 83% relative humidity and a similar value in solution at the same relative humidity. If this relation between vapor pressure and hydration in solution is correct then the hydration of a protein should not be independent of the environment. The assumption that the reference substance does not combine with the protein appears to be erroneous, because the salt concentration in certain protein crystals has been found to be greater than in the suspending medium in dilute salt solutions.[20,21] The combination of protein crystals with salt tends to give lower values for hydration in solution than are obtained from the vapor phase water uptake; in fact, the values for hydration in solution may appear to be negative.[20,21]

The conclusion that the specific volume of β-lactoglobulin in the hydrated crystal is 0.772 instead of the value of 0.751 based on a density of 0.99707 for water may also be true for β-lactoglobulin in solution. Adair and Adair[22] have found that there is no contraction in volume when edestin and hemoglobin crystals are dissolved. Assuming that water has a density of 0.99707, the apparent specific volume of β-lactoglobulin in the crystal is 0.753, as compared with 0.751 in solution, indicating a very small contrac-

tion on dissolving. Values of 1.003 and 0.752 for the specific volumes of water and protein, respectively, however, are obtained from density measurements on dilute solutions of β-lactoglobulin by the graphical intercept method rather than the values of 0.983 and 0.772 obtained on β-lactoglobulin crystals.

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Synopsis

The densities and water contents of large β-lactoglobulin crystals were determined as a function of relative humidity. When the specific volumes of the crystals (1/density) were plotted against their water contents, a straight line was obtained between 14 and 46% water. The extrapolation of this line by the graphical intercept method of Lewis and Randall gave a value of 0.772 for the specific volume of the protein and 0.983 for the specific volume of the water in the β-lactoglobulin crystal. Subtracting the specific volume of the protein in the crystal from the specific volume of the anhydrous protein (0.802 − 0.772) gave a value of 0.030 ml. for the contraction of one gram protein on becoming hydrated.

A value of 0.021 ml. was calculated for the contraction of water per gram protein by the difference between the apparent specific volume of the protein in solution, assuming water to have a specific volume of 1.003, and the value of 0.772 for the specific volume of the protein in the crystal. There was good correlation between the value for contraction

of water as calculated from the ionic groups of the protein and the observed contraction value.

Résumé

La densité et la teneur en eau de grands crystaux de β-lactoglobuline furent déterminés en fonction de l'humidité relative. En dessinant les volumes spécifiques (1/densité) des crystaux par rapport à leur tenure en eau, une ligne droite fut obtenue entre 14 et 46% d'eau. L'extrapolation de cette ligne par la méthode graphique d'interception de Lewis et Randall donna 0,772 pour le volume spécifique de la protéine et 0,983 pour le volume spécifique de l'eau dans le crystal de la β-lactoglobuline. Par subtraction du volume spécifique de la protéine dans le crystal de celui de la protéine enhydre (0,802 − 0,772) il résulta une valeur 0,030 ml. pour la contraction d'un gramme de protéine causée par l'hydratation.

On a calculé 0,021 ml. pour la contraction de l'eau par gramme protéine au moyen de la différence entre le volume spécifique apparent de la protéine en solution, admettant 1,003 comme volume spécifique de l'eau, et la valeur 0,772 du volume spécifique de la protéine dans le crystal. Il y avait un bon accord entre la valeur de contraction de l'eau calculée d'après les groupes ioniques de la protéine et celle obtenue par voie expérimentale.

Zusammenfassung

Dichte und Wassergehalt von grossen β-Lactoglobulinkrystallen wurden in ihrer Abhängigkeit von der relativen Feuchtigkeit untersucht. Aufzeichnung der specifischen Volumina (1/Dichte) der Krystalle gegen ihren Wassergehalt lieferte eine gerade Linie zwischen 14 und 46% Wasser. Deren Extrapolation mittels der graphischen Schnittmethode von Lewis und Randall ergab 0,772 für das specifische Volumen des Proteins und 0,983 für das des Wassers im β-Lactoglobulinkrystall. Durch Subtraktion des specifischen Volumens des Proteins im Krystall von dem des wasserfreien Proteins (0,802 − 0,772) fand sich 0,030 ml. als Kontraktion von 1 Gramm Protein bei der Hydratation.

0,021 ml. wurde berechnet für die Kontraktion von Wasser pro Gramm Protein aus der Differenz zwischen dem scheinbaren specifischen Volumen des gelösten Proteins—unter der Annahme eines specifischen Volumens 1,003 von Wasser—und dem Werte 0,772 des specifischen Volumens von Protein im Krystall. Es bestand gute Uebereinstimmung zwischen dem Kontraktionswerte des Wassers, wie er sich aus den Ionengruppen des Proteins berechnet, und dem beobachteten Kontraktionswerte.